

Tensile stress on microtubules facilitates dynein-driven cargo transport

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ABSTRACT: Mechanical stress significantly affects the physiological functions of cells, including tissue homeostasis, cytoskeletal alterations, and intracellular transport. As a major cytoskeletal component, microtubules respond to mechanical stimulations by altering their alignment and polymerization dynamics. Previously, we reported microtubules may modulate cargo transport by one of the microtubule-associated motor proteins, dynein, under compressive mechanical stress. Despite the critical role of tensile stress on many biological functions, how the tensile stress on microtubules regulates cargo transport is yet to be unveiled. The present study demonstrates that the low-level tensile stress-induced microtubule deformation facilitates dynein-driven transport. We validate our experimental findings using all-atom molecular dynamics simulation. Our study may provide important implications for developing new therapies for diseases that involve impaired intracellular transport.

KEYWORDS. *Mechanical stress, In vitro, Microtubule, Cargo transport, Dynein*

Microtubules are essential in many cellular processes, for example, cell shape regulation, cell division, intracellular transport, etc.^{1–3} The integrity of microtubules plays a vital role in these processes.⁴ Microtubules can also influence chemotherapeutic stressors and thereby regulate cancer cell survival.⁵ Therefore, the effect of mechanical stress on microtubules and neurons has been extensively studied. These studies suggest that microtubules contribute to sense cellular stress responses.^{6,7} Mechanical stress can alter polymerization dynamics,⁸ mechanical properties,⁹ growth,¹⁰ rescue, and self-healing of microtubules.¹¹ Mechanical stress even affects the microtubule-associated vesicle transport in neurons.¹² At extreme conditions, for example, in traumatic brain injury, microtubule rupture may take place.^{13,14} But how the cargo transport is maintained in such an adverse environment has remained elusive. In our present study, we address the effect of tensile stress on in vitro cargo transport. We investigate how the mechanical deformation of a microtubule due to external tensile stress affects the in vitro cargo transport by a microtubule-associated motor protein, dynein, using quantum dots (QDs) as cargo. We deform the microtubule by applying tensile stress on an elastic medium, which serves as the substrate for the microtubules.^{15,16} Our results show that stretching of microtubules facilitates the dynamics of dynein-driven transport along the microtubule. The enhanced dynein-based transport dynamics was also observed at low-level compression on microtubules in our previous study.¹⁷ To obtain a molecular-level insight, we used the all-atom molecular dynamics (MD) simulation

to investigate the interaction energies between dynein and microtubule constituting units, $\alpha\beta$ -tubulin heterodimer. Combining our experimental and simulation results, we achieve a better understanding of microtubules' mechano-biochemical responses under tensile stress. This work confirms the connection between microtubule deformation and the modulation of cargo transport.¹⁸ Our results could lead to new ways to treat diseases caused by microtubules or dynein-transport dysfunction and develop drugs targeting these mutations.^{19,20}

Deformation of microtubules by tensile stress. We used the 'micro-stretcher' developed previously to cause microtubule deformation (Figure 1a).¹⁶ We used an elastomeric substrate, polydimethylsiloxane (PDMS), as the substrate for fixing the microtubules. First, the PDMS was fixed and stretched by the micro-stretcher to eliminate any wrinkles. This was the initial condition for the microtubules. Then, kinesin, followed by microtubules prepared from fluorescently labeled tubulins, was introduced into the flow cell by shear flow. Flowing the microtubule containing buffer solution from one end of the channel and drawing it using soaking paper from the other end ensured horizontal alignment of the microtubule parallel to the stress axis. The adsorbed kinesins on the PDMS were the anchors to immobilize the microtubules.¹⁶ The concentration of kinesin was fixed to be 100 nM, in consistence to our previous study.¹⁷ No adenosine triphosphate (ATP) was present at this point.

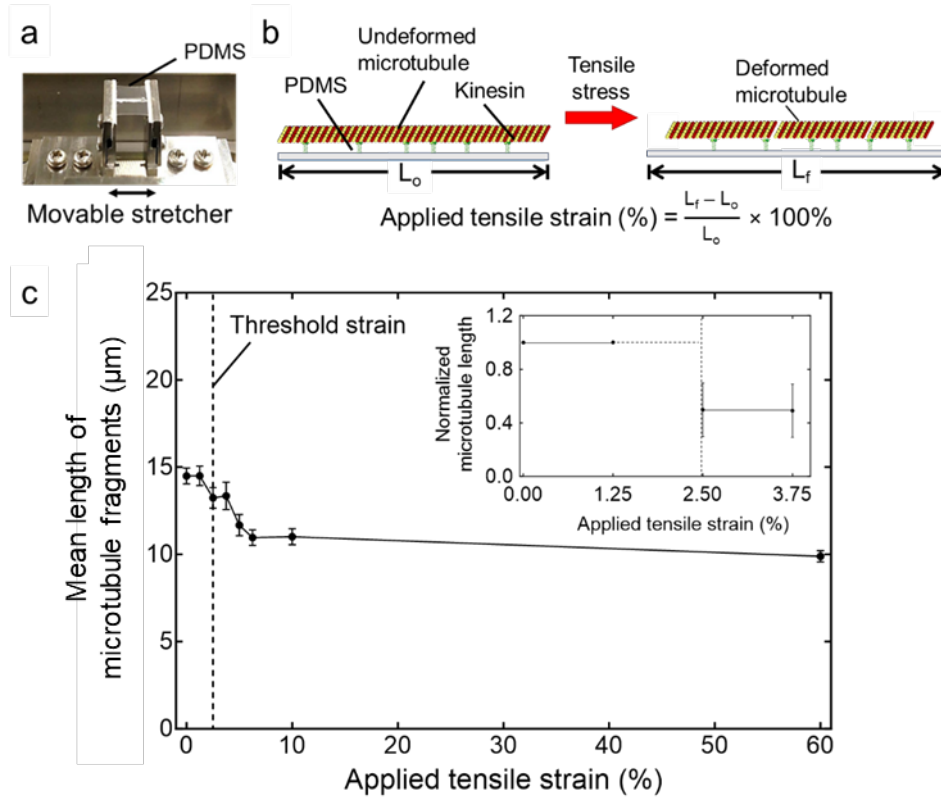


Figure 1. Microtubule deformation by tensile stress. **(a)** The micro-stretcher unit used for holding the PDMS substrate. The stress axis is along the horizontal axis. **(b)** Schematic images showing how the applied tensile strain is calculated using the initial length, L_o and final length, L_f of PDMS before and after applying tensile stress, respectively. **(c)** Mean lengths of the microtubules or microtubule fragments at different applied tensile strain. The vertical dashed line indicates that the threshold strain where the first cracking in microtubules becomes visible is at 2.50%. The number of microtubules or fragment of microtubules analyzed for this plot are between 30 to 115. Error bar: standard error. Normalized microtubule fragment lengths for one representative microtubule at low-level tensile strains are shown in the inset. At 2.50% strain the microtubule broke into 2 fragments, hence the error bar that represents standard deviation. Fluorescence images of the microtubule analyzed for this plot are given in Supporting Figure 1.

The microtubules attached to the PDMS through interaction with kinesin underwent tensile strain when the PDMS was stretched (Figure 1b). At a fixed kinesin concentration, stretching the PDMS at a fixed strain rate caused the microtubule immobilized on it to undergo fragmentation. (Supporting Figure 1).¹⁵ We confirmed to eliminate any possible damage by PDMS on microtubule-kinesin interaction by purging humid nitrogen to the micro-stretcher before and during the observation which was verified by the non-significant difference between the mean instantaneous gliding velocities of the microtubules on kinesin-coated glass and PDMS substrates (Supporting Figure 2).²¹ We checked the lengths of the microtubules or their fragments at each tensile strain (Figure 1c). The average length of the microtubules investigated in this study was ~15 μm . The first crack visible in the fluorescence microscope images appeared at a tensile strain equal to 2.50%, evident from the mean length values shown in Figure 1c. This strain has been defined as the threshold strain in literature.¹⁵ The strain was applied at a rate of 0.44 s^{-1} for all cases.

Dynein-driven cargo transport along microtubules deformed by tensile stress. We investigated the effect of tensile stress on the microtubule-associated cargo transport, by observing the multiple dynein-driven quantum dot (QD) transport along the microtubules deformed due to applied tensile strain. We conjugated dyneins with SNAP tag to the benzyl guanine (BG)-functionalized QDs (10 nM

dynein mixed with 1 nM QD).²² In our system, three or fewer dynein molecules were bound with one QD, as estimated previously.²³ For the preparation of the experiment assay, the microtubules were anchored by kinesins on PDMS, and tensile stress was applied as described in the previous section. We applied tensile strains at 1.25% intervals because we assume in ideal case, 1.25% stretching of the 8 nm tubulin dimer will increase the length by 0.1 nm, similar to what was reported previously.²⁴

After the tensile stress was applied on the microtubules, we introduced a long-practiced method of glutaraldehyde treatment followed by quenching by glycine to confirm the microtubules do not glide on the anchor kinesins in the presence of ATP.^{25–27} While alternative methods like rigor-binding kinesin motors²⁸ or anti-tubulin antibodies²⁹ could also be promising approaches, we used 0.1% (v/v) glutaraldehyde consistent to our previous works which has already been reported by our group³⁰ or others³¹ to not cause a detrimental effect on microtubules in terms of microtubule to motor protein interaction. We also confirmed the non-significant effect of 0.1% (v/v) glutaraldehyde on microtubule-motor protein interaction by comparing the gliding assays of 0.1% (v/v) glutaraldehyde-treated microtubules to untreated microtubules as shown in Supporting Figures 3 and 4. Finally, the dynein-QD conjugates in the presence of 1 mM ATP were introduced to transport along the deformed microtubules at different tensile strains (Figure 2a).

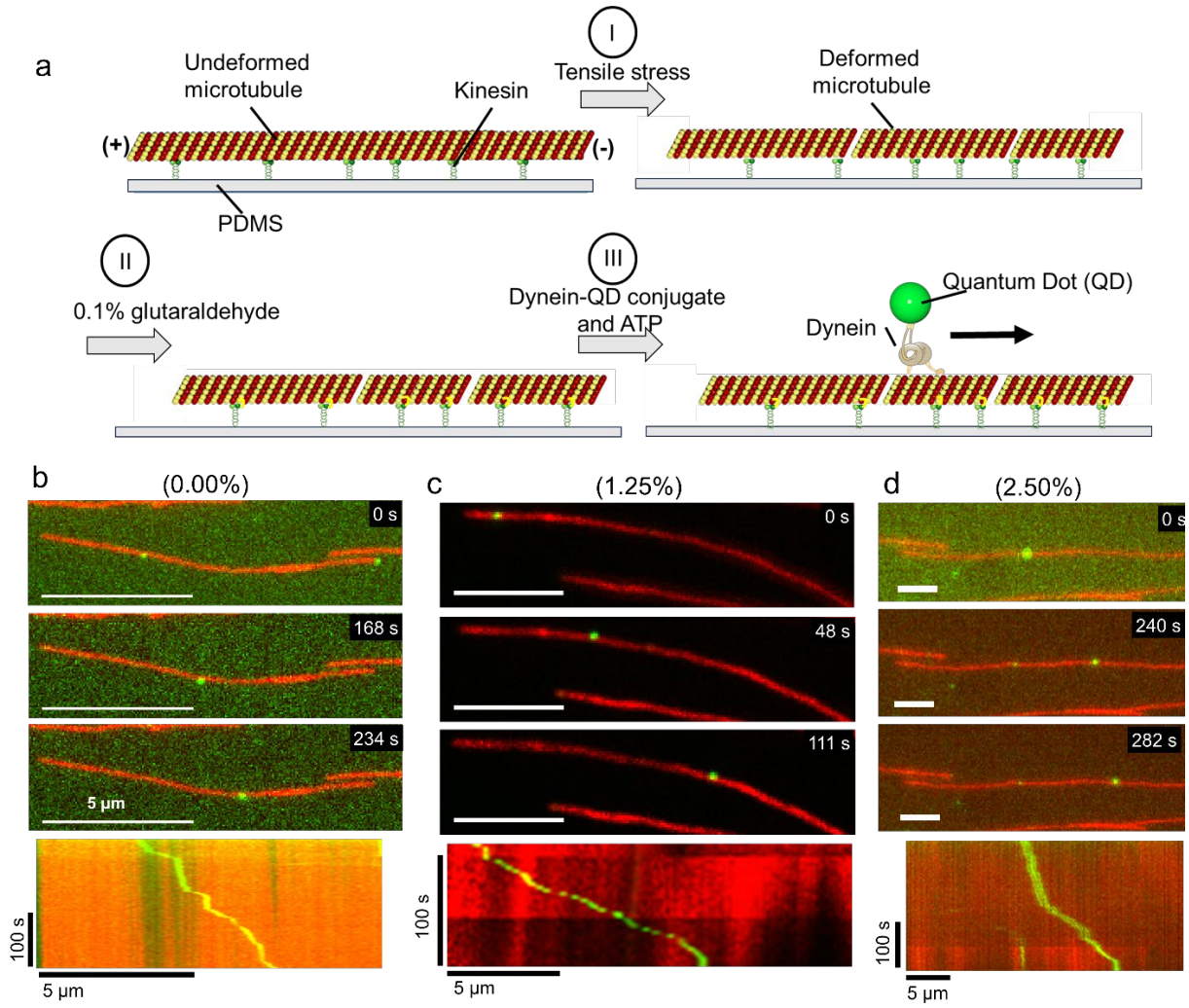


Figure 2. Observation of QD transport by dynein along microtubules deformed due to tensile stress. **(a)** Schematic showing the assay of QD transport by dyneins along a microtubule deformed by applying tensile stress. To observe QD transport along microtubules deformed by tensile stress, the PDMS substrate was stretched so that microtubules underwent tensile strain. After that, glutaraldehyde was added to immobilize microtubules. Finally, dynein-QD conjugate was introduced in the presence of ATP. **(b-d)** Time-lapse fluorescence images depicting dynein-driven QD transport along microtubules under various applied tensile strains as indicated in the parenthesis. Scale bar: 5 μm . The kymographs of the respective events are shown in the bottom panels. Scale bar: 5 μm and 100 s for horizontal and vertical axes, respectively.

The fact that glutaraldehyde was introduced after applying the tensile stress confirms any alteration in dynein-driven transport resulted from tensile stress induced deformation of microtubules. Multiple dynein conjugated cargos transport unidirectionally towards the microtubule minus end *in vitro*.^{32,33} We could observe the dynein-based QD transport along the stretched microtubules at applied tensile strains equal to 1.25%, 2.50%, 3.75%, and 5.00% (Supporting Figure 5). At tensile strain higher than 5.00%, we observed attachment of QDs on microtubules, but no QD transport by dynein was observed. Time-lapse fluorescence images of QD transport by dynein along microtubules under different tensile strains from 0% to 5.00% are given in Figures 2b-d.

Time-displacement plot of the dynein-driven QDs along microtubules deformed by different tensile strains are shown in Figure 3a. The time-displacement plots of the dynein driven QDs show two typical behaviors of the velocities that include a combination of fast states and slow states of movements, typical of dynein behavior.³⁴

Instantaneous velocities of each moving QDs were calculated and their distribution were plotted in Supporting Figure 6. We found that dynein exhibited pauses while transporting QDs along microtubules.^{17,35} Therefore, we determined arithmetic means of the velocities including or excluding the pausing events and presented against the applied tensile strain as shown in Figure 3b. The mean velocity of QD transport by dynein along undeformed microtubules was $43 \pm 7 \text{ nm s}^{-1}$ (mean $\pm$ standard error, number of events, $n=44$), which agrees to previous reports.^{17,32,36} We observed the velocities of the dynein-driven QDs increased with an increase in tensile strain up to 2.50%, which is the threshold strain that induces breakage in the microtubules. From over 2.50% until 5.00% applied tensile strain the mean velocities decreased. At 6.25% strain, we observed no motility events. Deformation of microtubules by tensile stress also increased the run length of dynein transporting the cargo (Figure 3c,d and Supporting Figure 7). We do not observe any significant increase in the pause frequency while transporting QDs by dynein along the microtubules (Supporting Figure 8).

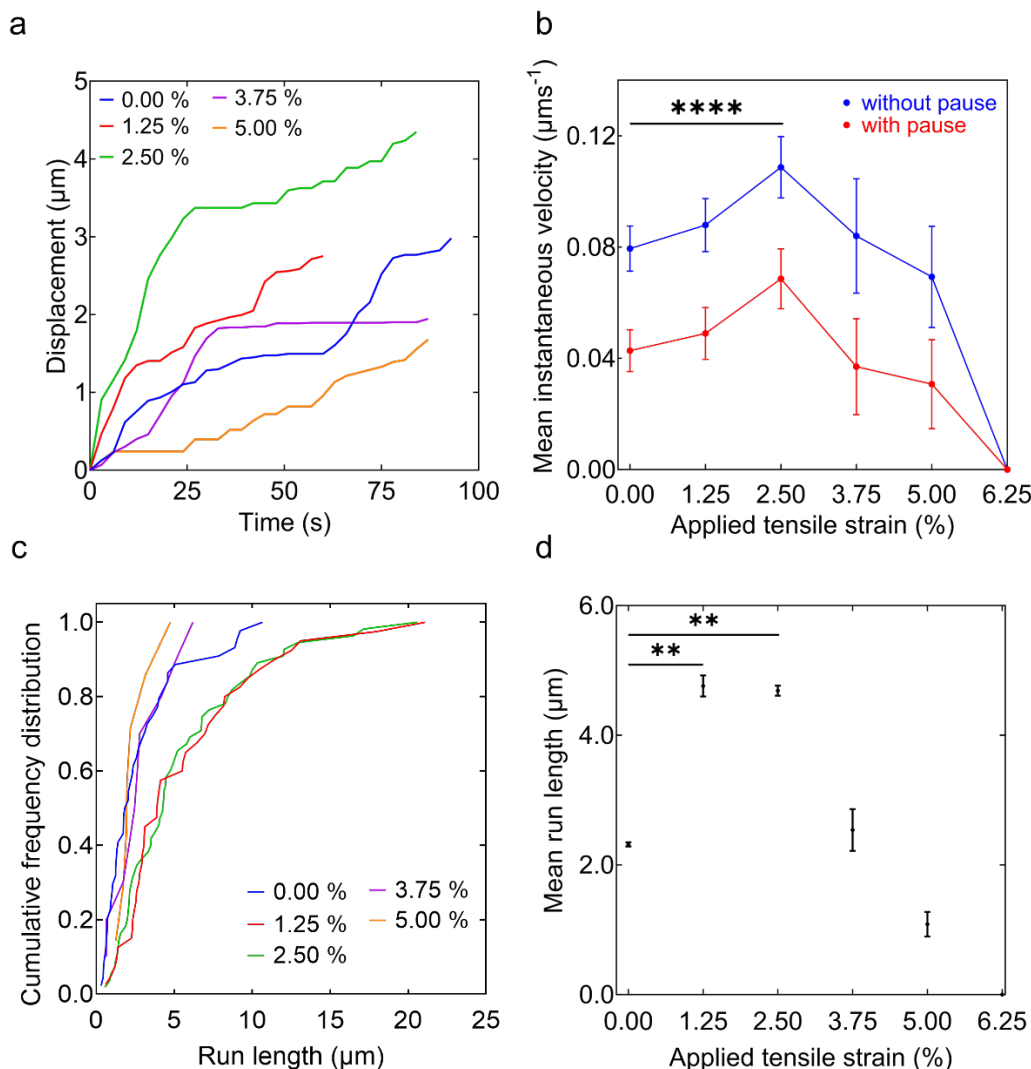


Figure 3. Response of dynein-driven transport along microtubules under tensile stress. **(a)** Time-displacement profiles of dynein-driven QDs while transporting along microtubules stretched by different tensile strains (0.0% to 5.00%). Representative events for each case are shown in the plot. **(b)** Mean instantaneous velocities of dynein-driven QDs as a response to the applied tensile strain on the microtubules. Red circles are arithmetic mean values calculated from velocities including pauses, and blue circles are for mean velocities without considering the pausing events. Tests for normal distribution gave negative results for the velocity data. The statistical significance of data was checked using Kruskal- Wallis non-parametric test and Dunn's multiple comparisons post hoc tests. ** $P < 0.001$, **** $P < 0.0001$ and ns=non-significant. The numbers of QDs analyzed for this plot and all results of the statistical analyses are available in Supporting Table 1. **(c)** Cumulative frequency distribution of dynein run lengths at different tensile strains. The number of events considered for this analysis are 44, 40, 55, 10 and 7 for the cases of 0.00, 1.25, 2.50, 3.75 and 5.00% tensile strains, respectively. **(d)** Mean run lengths of dynein transporting QDs along microtubules under different applied tensile strains. We used the bin-independent cumulative probability distributions to determine the mean run lengths of dynein-driven QD along undeformed and deformed microtubules. See also Supporting Figure 7 and Supporting Tables 2 and 3. Error bar: standard error in all figures.

MD simulations for understanding the effect of the deformation of microtubule on the molecular interaction between dynein and tubulin. We verified the relationship between the microtubule deformation and dynein-deform microtubule interaction from an energetic aspect by using computer simulations.³⁷ We used all-atom MD simulations of a protein complex system using initial structure from Protein Data Bank (PDB) ID: 6RZB. We use this model for the high-affinity microtubule-binding domain (MTBD) crystal structure of dynein,³⁸ as we are interested in investigating the attachment dynamics of dynein with microtubules with or without deformation. Our model includes the dynein MTBD, one tubulin dimer representing the microtubule, and surrounding water

molecules (Figure 4a). Hydrogen atoms were added computationally. We calculated the intermolecular interaction energies by adding electrostatic and van der Waals and interaction energies between the dynein MTBD and the tubulin dimer at different center-of-mass (COM) distances. The values of the electrostatic and van der Waals interaction energies were evaluated by Coulomb interaction energy and Lennard-Jones interaction energy, respectively.

The interaction energy was averaged over all the structures sampled by our MD simulation.^{37,39–41} We found that the total intermolecular interaction between the proteins decreased when the inter-tubulin distance was altered, either there was an increase or decrease in

the tubulin-tubulin distance (Figure 4b and Supporting Figure 9). This means the interaction of the dynein motor domain is weaker with tubulin deformed by either compression or tension.

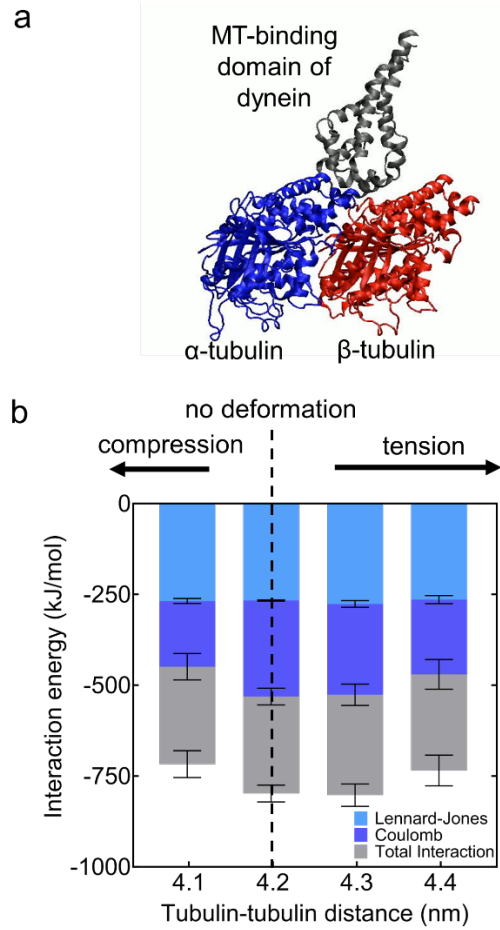


Figure 4. MD simulation study of dynein MTBD-tubulin interaction at different tubulin-tubulin distances. **(a)** MD simulation model used in this work. MT= microtubule. **(b)** The total interaction energy between the dynein MTBD with the tubulin dimer with varying center-of-mass distances between α -tubulin and β -tubulin. The values of the interaction energies are superimposed on each other. Total interaction energy is (grey bars) is the sum of Lennard Jones (light blue bars) and Coulomb (dark blue bars) interactions. The vertical dashed line shows the inter-tubulin distance when there is no deformation in a microtubule. Five runs were averaged to obtain the mean values. Error bars are standard errors.

Dynein has been reported to efficiently overcome any obstacles on the track during its motion along the microtubules.⁴² It can switch steps to adjacent protofilaments, which may help them overcome the defect in the microtubule.⁴³ Additionally, the structural flexibility⁴⁴ and robust behavior^{23,45} of dynein may favor its motility along the deformed tracks. Previously, we reported accelerated dynein-based QD transport by compression on the microtubules.¹⁷ The stretching of the microtubules on the elastic PDMS can be translated to an increase in the tubulin-tubulin spacing among the constituting tubulin dimers of microtubule filaments. The increase in the spacing has somewhat favored the dynein motility as apparent from the significant increase in mean velocities of the dynein-

driven QDs with the increment in the applied tensile strain at low levels. It is intuitive because of the tension regulated stepping of dynein i.e., when the separation between the heads gets wider on the microtubule increasing the intramolecular tension in dynein, the dwell time before the next step is shortened and the probability of the rear head advancing increases and thus the velocity increases with longer run length.^{46,47} The stretching of the microtubules might influence the dynein run length in a complex way. As we observe from the MD simulation, an increase in the tubulin-tubulin distance of microtubules caused decreased interaction energy with dynein suggesting an increased detachment rate of dynein motors. The slower detachment rate may cause shorter but more frequent stepping and thus potentially lead to longer run lengths with faster velocities referred as long-fast travel states of dynein in literature.³⁴ In our MD simulations, we alter the α -tubulin and β -tubulin COM distance by 0.1 nm in analogy to extension or compression of the microtubule, and we observe that either deformation caused decrease in the intermolecular interactions between the tubulin dimer and the dynein MTBD. Our MD simulation environment involves COM distance up to 4.4 nm which is the length of tubulin dimer caused by 2.5% tensile stress, in ideal case. We observe a more pronounced decrease in the interaction energy between tubulin dimer and the dynein MTBD aligning with the increase observed in the velocities of the dynein-driven QDs at 2.50% tensile strain in Fig. 3b. The decrease in interaction energy would lead to faster detachment, with more frequent steps and higher velocity, making this finding consistent with the experimental results within this range.⁴⁸ The affinity of dynein for microtubules is modulated by long-range electrostatic interactions, and specific salt bridges and dynamic interactions play a significant role in regulating the binding affinity of dynein for microtubules.⁴⁹ Additionally, the communication between the AAA+ ring and the MTBD of dynein is influenced by the distribution of hydrophobic and hydrophilic residues, further affecting the interaction with microtubules.⁵⁰ Therefore, tubulin dimers of stretched microtubules with enhanced exposure of their hydrophobic core will result in weaker of electrostatic interactions between the dynein MTBD and deformed microtubules as observed in the MD simulation results. Dynein has a stochastic stepping mechanism with variable step sizes ranging from 8 nm to 40 nm.^{46,47} Larger step sizes, mostly 24 and 32 nm, are reported not only in adverse environments, for example, in the presence of high loads or at low ATP levels, but also in the absence of external load.⁵¹ The presence of a 14 nm-long stalk and flexible joint of dynein might allow it for a larger displacement of the MTBD and successful transport along microtubules deformed by tensile stress even higher than the threshold strain of 2.5%, although at a lower velocity.^{52,53} The threshold strain differentiates between the elongation and breakage of the microtubules, similar to that reported for axons in cells.^{14,54} Increase in microtubule deformation above this strain caused decreased QD attachment as evident from the low number of events available at 3.75% and 5.00% tensile strain. However, the theoretical explanation for the maximum velocity occurring at 2.5% tensile strain remains unclear with the current scope of our study and warrants further investigation.

Finally, at 6.25% tensile strain, we do not observe any motility by dynein, meaning that, although dynein overcome increased spacing between dimers due to large step sizes the motility is hindered,

possibly because during the stepping of the two dynein heads, one motor domain can step two or three times in a row without the second motor domain steps.⁵⁵

In conclusion, we systematically investigated the response of microtubule functionality in cargo transport by employing a simple in vitro system. Our results show that for tensile strains $\leq 2.50\%$, the dynein-driven transport was facilitated while higher strain up to 5.00% delayed their motion, and finally, over 5.00% tensile strain, the transport was halted entirely. The dynein velocity along microtubules in the presence of ATP should be determined by four factors: ATPase rate, stroke size, detachment rate, and attachment rate. If either the detachment rate or attachment rate of dynein increases, the velocity also increases. From the MD simulation, we observe the increased detachment rate due to an increase in the tubulin-tubulin distance, which would result in frequent stepping at a given attachment rate and thus increase the velocity of dynein-driven transport. However, in our current range of experiments, we cannot determine the role of attachment rate, and more research is needed to definitively explore this concept and its impact on dynein processivity. The modified dynamics of dynein motor-driven transport observed in our study could also serve as a foundation for other regulatory mechanisms, such as the control of cellular activities through post-translational modifications of tubulins.⁵⁶ Tensile stress plays modulating roles in cellular mechanosensing and intracellular transport on microtubules as it stimulates microtubule outgrowth.^{57,58} Thus, the facilitated dynein driven transport along microtubules under tensile stress may be a strategy to the cellular adaptation process on how to respond to external mechanical stimuli. Understanding of how microtubule deformation influence dynein motility may be employed to identify novel therapeutic targets or strategies and develop targeted interventions. This could offer remedies to diseases characterized by impaired cargo transport such as neurodegenerative disorders, cancer, and other rare genetic conditions.^{59,60}

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at

Additional experimental details, materials and methods, including purification and dye labeling of tubulin and polymerization of microtubules, expression and purification of motor proteins, preparation of PDMS film, experimental design: QD transport assay by dynein, preparation of dynein-QD conjugate, experimental design: gliding assay of microtubules on the kinesin-coated glass substrate, experimental design: gliding assay of untreated microtubules and glutaraldehyde-treated microtubules on the motor protein-coated glass substrate, experimental design: gliding assay of microtubules on kinesin-coated PDMS substrate, microscopy image capture and data analysis, estimation of mean velocity, run length, and pause frequency of dynein, MD simulation, statistical analysis, nine supporting figures showing the effect of tensile stress on a representative microtubule, effect of PDMS on the microtubule gliding velocities on kinesin-coated surface, distributions of the instantaneous gliding velocities of microtubules, effect of treatment of microtubules with 0.1% GA on the microtubule-motor protein interaction, trajectories of the moving QDs by dynein along microtubules, distributions of the instantaneous velocities of dynein-driven QDs along microtubules stretched under different tensile strains,

cumulative frequency distributions of dynein run length at different tensile strains, effect of applied tensile strain at microtubules on the pause frequency of dynein-driven cargo transport along the microtubules, the interaction energies between each of the tubulin monomers and dynein MTBD at different tubulin-tubulin distances, three supporting tables including results of the normality test and the statistical analysis of the velocities, and run lengths of the dynein-driven QDs along microtubules at different applied tensile strains, fitting information of the cumulative probability distribution of run lengths, and references for supplementary information.

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Author Contributions

Conceptualization: SRN, Methodology: SRN, TY, TT, Investigation: SRN, TY, Visualization: SRN, Resources: TY, TT, KO, AK Supervision: AK, Writing—original draft: SRN, Writing—review & editing: SRN, TY, MI, TT, KO, KS, AK. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest. All data are available in the main text or the Supporting information.

ABBREVIATIONS

QD, Quantum dot. PDMS, polydimethylsiloxane. MD, Molecular dynamics. MTBD, Microtubule Binding Domain.

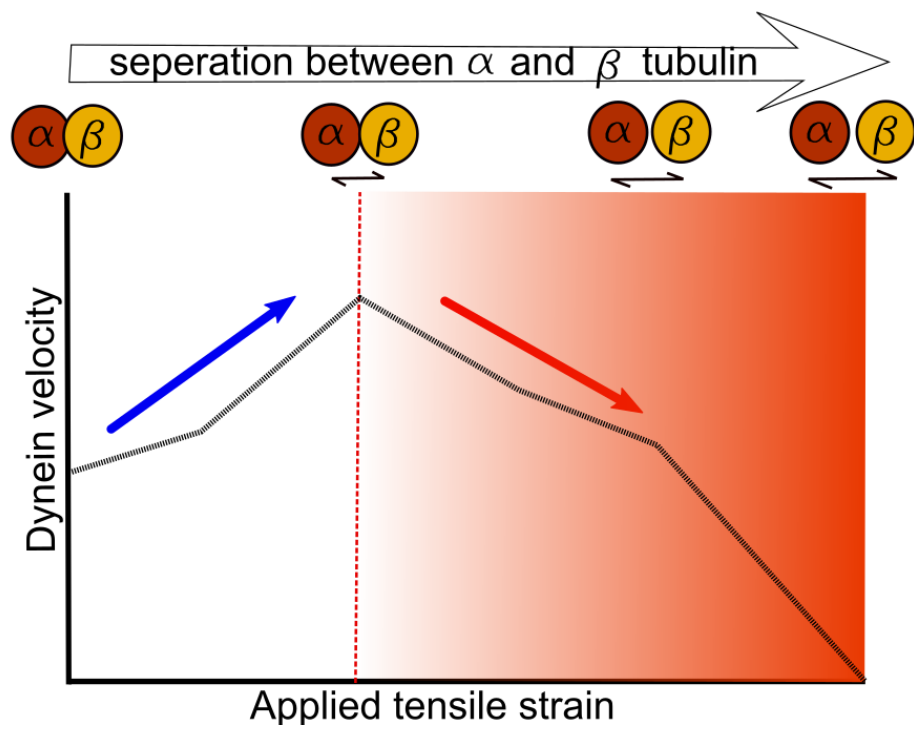
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Supporting Information

Tensile stress on microtubules facilitates dynein-driven cargo transport

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MATERIALS AND METHODS

Purification and dye labeling of tubulin and polymerization of microtubules. The tubulin used for microtubule polymerization was purified from fresh porcine brain using a high-concentration PIPES buffer (1 M PIPES, 20 mM EGTA, 10 mM MgCl₂; pH adjusted to 6.8 using KOH).¹ Rhodamine-labeled tubulin was prepared using 5/6-carboxy-tetramethyl-rhodamine succinimidyl ester (TAMRA-SE; Invitrogen).² The absorbance of tubulin at 280 nm and tetramethyl-rhodamine at 555 nm showed that the tubulin-to-dye labeling ratio was 1.0. A mixture of four parts of labeled and one part of non-labeled tubulin with a final tubulin concentration of 55.6 μ M was polymerized at 37 °C in the presence of 5 mM GTP, 20 mM MgCl₂, 25% DMSO in BRB80. The microtubule was paclitaxel-stabilized (50 μ M paclitaxel in BRB80; pH 6.8).

Expression and purification of motor proteins. A recombinant conventional kinesin-1 construct consisting of human kinesin (residues 1-573) was expressed and purified to serve as anchors to fix microtubules on PDMS and to perform the microtubule gliding assay experiments on kinesin-coated substrate.³ Full-length human cytoplasmic dynein with sequence *SBP-SNAP-GGGSTR-DHC* was purified according to the literature.⁴

Preparation of PDMS film. We prepared the PDMS film by mixing the precursor (Silpot 184[®], Dow Corning Toray Co. Ltd.) and cross-linker (Catalyst of Silpot 184[®]) at a 10:1 (w/w) ratio and diluting it to 1:10 (w/w) using hexane. After shaking and degassing in a vacuum for an hour, the mixture was spin-coated on a Teflon[®] sheet at 1500 rpm for 30 seconds to ensure even coating. The spin-coated sheets were cured at 60 °C for 4-6 hours to allow polymerization into PDMS and complete evaporation of hexane. The thin PDMS film was peeled off from the Teflon[®] sheet to use in an experiment.

Experimental Design: QD transport assay by dynein. *In vitro* QD assays along microtubules were performed on PDMS using the micro-stretcher.^{5,6} It contains a cover plate and a base plate with a computer-controlled stretcher/compressor. First, we set the distance between the two clumps to 4.0 mm and fixed a piece of PDMS (L×W×T ~ 8.0×5.0×0.05 mm) on the stretcher which was then extended to 8.0 mm to avoid any wrinkles. This length of 8.0 mm is the initial

length for calculating the tensile strain and this condition is the unstretched condition for the microtubules. The elongated PDMS was masked with two 18×18 cover glasses, leaving a narrow channel in the middle as a flow cell. This channel was plasma etched (SEDE-GE; Meiwafoysis Co. Ltd.) for 4 minutes (10 Pa, 8 mA) to make the substrate hydrophilic. 10 μ L of 100 nM kinesin in buffer A (~80 mM PIPES, 1 mM EGTA, 1 mM MgCl₂, 1 mM dithiothreitol, 10 mM paclitaxel; pH 6.8) was introduced to the flow cell and incubated for 5 minutes. The flow cell was washed with buffer B (~80 mM PIPES, 1 mM EGTA, 1 mM MgCl₂, 0.5 mg/ml casein, 1 mM dithiothreitol, 10 mM paclitaxel; pH 6.8). Next, paclitaxel-stabilized, rhodamine-labeled 280 nM 20 μ l microtubule solution was introduced and incubated for 5 minutes, followed by washing with buffer B. The shear flow of the microtubule solution from one end of the flow cell ensured the parallel alignment of microtubules to the stretch axis. The microtubules were immobilized by exposure to 0.1% (v/v) glutaraldehyde for 4 minutes. The glutaraldehyde fixing reaction was quenched by 0.1 M glycine and incubated for 5 minutes. Finally, the dynein-QD conjugates (details of preparation given later) were introduced into the flow cell. The chamber was then sealed, and humid nitrogen gas was purged for 30 minutes before the observation in a fluorescence microscope. The humid nitrogen purging continued until the end of the observation to create an inert atmosphere for the assay.⁷ All experiments were performed at 25 °C.

Preparation of dynein-QD conjugate. The QD (Qdot® 525 ITK™ Carboxyl Quantum Dots, Molecular Probes) used in this study was functionalized with benzylguanine (BG) so that it covalently binds to the SNAP-tagged dynein motors through BG-SNAP interaction.⁸ To obtain QD-dynein conjugates, dynein, and QD were mixed at a molar concentration ratio of 1:10 and incubated at room temperature for 30 minutes. The applied concentrations of QD and dynein to the flow cell were 1 and 10 nM, respectively as diluted by ATP buffer (1 mM ATP, 1% Pluronic F127, 1 mg/ml casein, 20 mM dithiothreitol, 10 μ M paclitaxel in BRB80, and assay buffer having 20 mM PIPES, 4 mM MgSO₄, 10 mM K-acetate, 1 mM EGTA; pH 6.8).

Experimental Design: gliding assay of microtubules on the kinesin-coated glass substrate. In order to perform the gliding assays of microtubules on kinesin coated substrate, a flow cell was prepared by making a narrow channel with double-sided tape on a 28 mm×50 mm glass slide covered with 18 mm×18 mm coverslip. The double-sided tape served as a spacer. The volume of

all solutions introduced to the flow cell was 5.0 μ l. 0.5 mg/ml casein in BRB80 was introduced to the flow cell and allowed to adsorb for 5 minutes. The flow cell was washed using the wash buffer comprising 0.5 mg/ml casein, 1 mM dithiothreitol, 10 μ M paclitaxel, and the oxygen scavenger system using 4.5mg/ml glucose, 50 unit/ml glucose oxidase, and 50 unit/ml catalase in BRB80 (pH 6.8) before addition of 800 nM kinesin. After allowing the kinesins to adsorb for 5 minutes on glass the flow cell was washed again and 0.02 mg/ml microtubules were passed. The microtubules were allowed a 5-minute incubation before ATP buffer containing 5 mM ATP in the presence of 0.5 mg/ml casein, 1 mM dithiothreitol, 20 μ M paclitaxel, and the oxygen scavenger system in BRB80 (pH 6.8) was applied. The flow cell was immediately observed in the fluorescence microscope to investigate the microtubule gliding on kinesin in the presence of ATP.

Experimental Design: gliding assay of untreated microtubules and glutaraldehyde-treated microtubules on the motor protein-coated glass substrate. In order to perform the gliding assays of microtubules on the motor protein-coated substrate, a narrow channel on glass was prepared according to the previous section. Glutaraldehyde-treated microtubules were obtained by incubating microtubules in the presence of 0.1% glutaraldehyde for 15 minutes. Untreated and glutaraldehyde-treated microtubule gliding assays on kinesin-coated glass substrates were performed separately following the experimental design described in the previous section. For the dynein-supported microtubule gliding assay observation, 1 mg/ml streptavidin was introduced to the flow cell and allowed to incubate for 5 minutes. 0.5 mg/ml casein was applied to the flow cell twice and incubated for 5 minutes again. 15 nM dynein was then introduced, incubated for 5 minutes, and washed using HEPES buffer. After that, microtubules, untreated or glutaraldehyde-treated were introduced to the flow cell in the presence of 5 mM ATP, 2 mM dithiothreitol 10 μ M paclitaxel, 50 mM K-acetate, 1 mg/ml casein, 25 μ M glucose, 50 unit/ml glucose oxidase, and 50 unit/ml catalase. The observation of the flow cell in the fluorescence microscope started within 5 minutes of the addition of microtubules.

Experimental Design: gliding assay of microtubules on kinesin-coated PDMS substrate. A narrow channel on the PDMS substrate fixed onto the micro-stretcher was prepared to use as a flow cell as explained in the section *Experimental Design: QD transport assay by dynein*. The microtubule gliding assay on kinesins was performed following the steps described in the

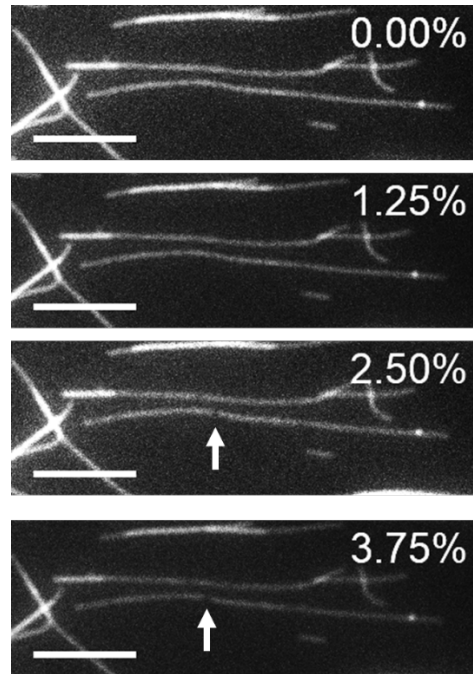
previous section. The micro-stretcher was then sealed, and humid nitrogen gas was purged for 30 minutes before the observation and continued until the end of the observation.

Microscopy image capture and data analysis. Samples were illuminated with a 100 W mercury lamp and visualized by a two-color epi-fluorescence microscope (Eclipse Ti; Nikon) equipped with an oil-coupled Plan Apo 60×1.40 objective (Nikon). We used filter blocks with UV-cut specifications (TRITC: EX540/25, DM565, BA606/55; Q525: EX435/40, DM510, 525/15; Nikon). We used a cooled CMOS camera (Neo CMOS; Andor) connected to a PC to capture images. Photobleaching was avoided by inserting ND4 (25% transmittance) for microtubules and ND1 (100% transmittance) for QDs into the illuminating light path of the microscope. The QD transport was observed by capturing images at a rate of 1 frame/3 s with an exposure time of 300 ms. All movies captured by the fluorescence microscope were analyzed using ImageJ. The trajectories of the QDs transported by the motors were tracked manually using an ImageJ plugin, MTrackJ.⁹ The QDs that could be traced on microtubules for three or more frames were tracked for analysis. For strain measurement, the length of pre-stretched PDMS was considered the initial length, and the percentage of the strain experienced by microtubules on PDMS was calculated.

Estimation of mean velocity, run length, and pause frequency of dynein. Instantaneous velocities were determined from the trajectories obtained using ImageJ. The mean values were the arithmetic means of the instantaneous velocities. Kymographs were obtained using the ImageJ plugin KymographResliceWide. To determine the mean run length (x), non-linear least-squares fitting of the cumulative probability distribution to the function ¹⁰: $Y = 1 - \exp(-\frac{(x_0 - x)}{t})$, was performed. x_0 is the lowest run length measured during analysis. The only fit parameter, the decay constant, t , gave the mean run length in the distribution. Pause frequency was calculated according to the literature where the total number of the pauses (at 3s time intervals the QD displacement $\leq 0.11 \mu\text{m}$) was divided by the total distance traveled by the QD (run length).¹¹ The QD displacement of $\leq 0.11 \mu\text{m}$ corresponds to the minimum pixel available in our experimental system.

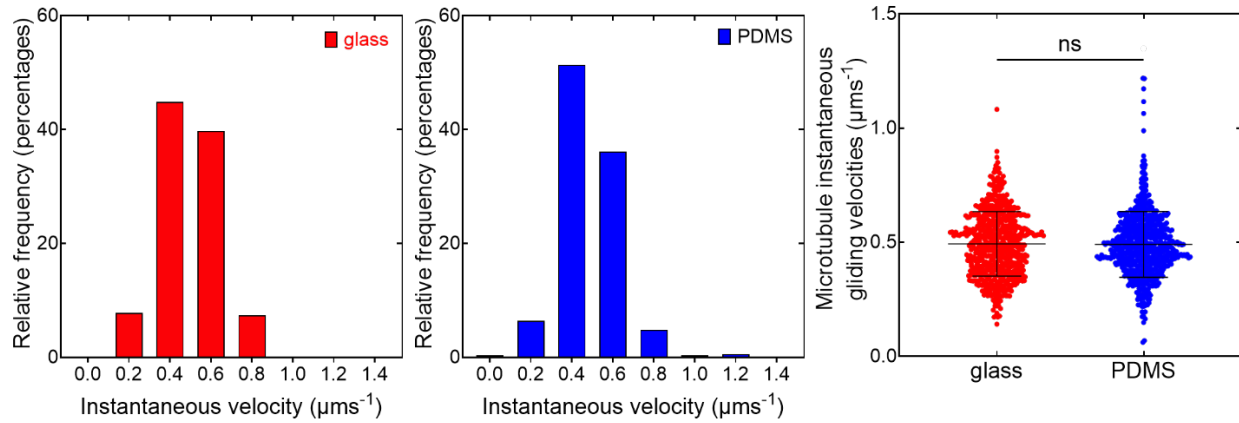
MD simulation. The dynein-tubulin system utilized in this study consisted of a tubulin-dynein complex, 69,918 water molecules, 239 Na⁺ ions, and 205 Cl⁻ ions. GDP and GTP molecules were located in the pockets of tubulin. The initial structure of the tubulin-dynein complex was taken from the Protein Data Bank (PDB) (ID: 6RZB). For the MD simulations, we used the GROMACS program (version 2019.4)¹² and the FUJI force field^{13–15} along with the TIP3P water model. First, the system was energetically minimized using the l-BFGS method. Then, an MD simulation was conducted for 300 ps with the heavy atom positions of the tubulin-dynein complex restrained. Next, we removed the position restraints, except for the stalk part (Ala3270-Pro3285, Met3403-Glu3418), and conducted a 600 ns MD simulation to equilibrate the system. As in a previous study, the distance between the center of mass (COM) of α -tubulin and that of β -tubulin was restrained with a harmonic potential in the production run to mimic the effect of the mechanical deformation of microtubules.¹⁶ The spring constant was set to 1000 kJmol⁻¹nm⁻², and the equilibrated distance was set to 4.1, 4.2, 4.3, and 4.4 nm in respective windows. In each window, five MD simulations were conducted for 210 ns. In all MD simulations, the Nose-Hoover coupling and Berendsen coupling were used to control the temperature (298 K) and pressure (1 bar), respectively. The time step was set to 3 fs. The long-range electrostatic interaction was calculated using the PME algorithm. All chemical bonds were constrained using the LINCS algorithm. We analyzed the interaction energy between the tubulin dimer and dynein, where the interaction energy was calculated as the sum of Lennard-Jones interaction and the Coulomb interaction and averaged over the last 150 ns of each MD simulation. The expected values and standard errors were calculated from the interaction energy values obtained in the five independent MD simulations.

Statistical analysis. Statistical analyses were performed using GraphPad Prism (www.graphpad.com). Student's t-test was performed to compare two groups of normally distributed data. Kruskal-Wallis test, followed by Dunn's multiple comparison test, was used to compare three or more non-normal data groups. The number of events, *n*, *P*-values, and name of the statistical test performed for each analysis are available in figure captions or Supplementary Information.



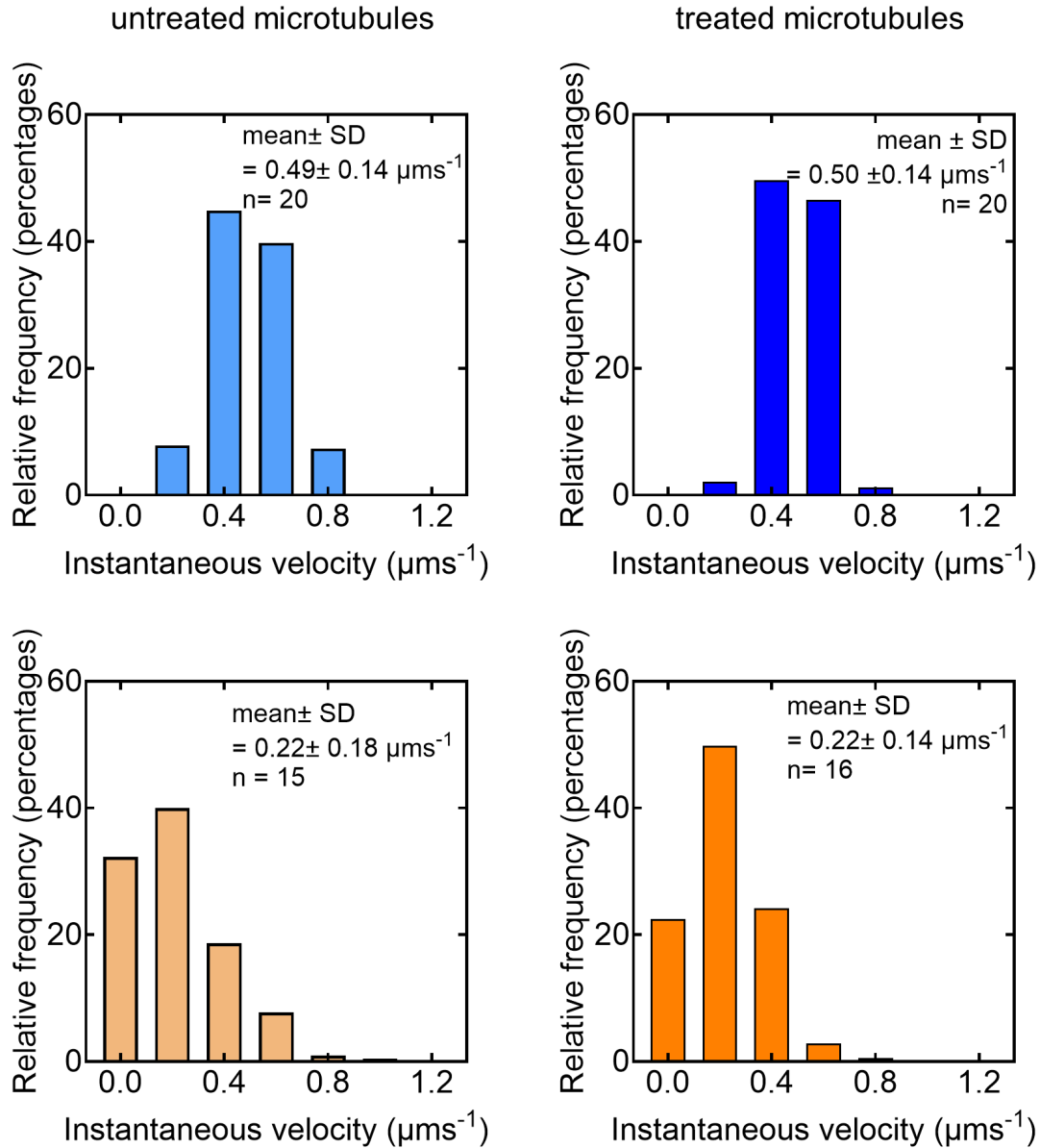
Supporting Fig. 1.

Effect of tensile stress on a representative microtubule. Fluorescent images of microtubules stretched by applying tensile stress used in the inset of Fig. 1c. Scale bar: 10 μm .



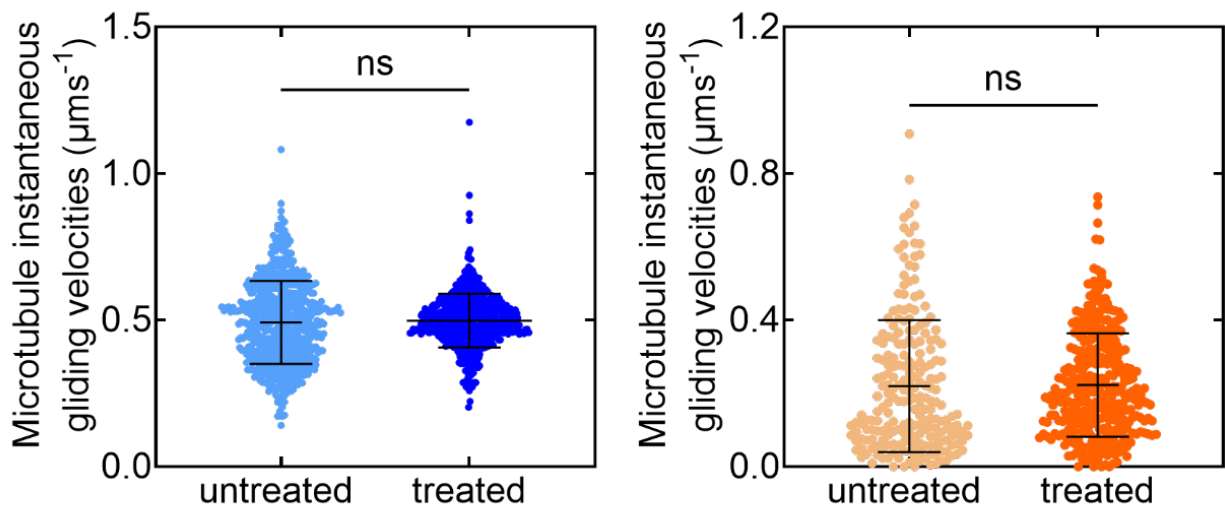
Supporting Fig. 2.

Effect of PDMS on the microtubule gliding velocities on kinesin-coated surface. Histograms of the microtubule instantaneous gliding velocities on kinesin-coated glass or PDMS substrates. Bin size = $0.02 \mu\text{m s}^{-1}$. The mean instantaneous gliding velocities of microtubules on kinesin-coated glass or PDMS are statistically non-significant as analyzed using Student's t-test, $P=0.7705$. ns = non-significant. 20 microtubules in each cases were analyzed for this analysis. Error bars: standard deviation.



Supporting Fig. 3.

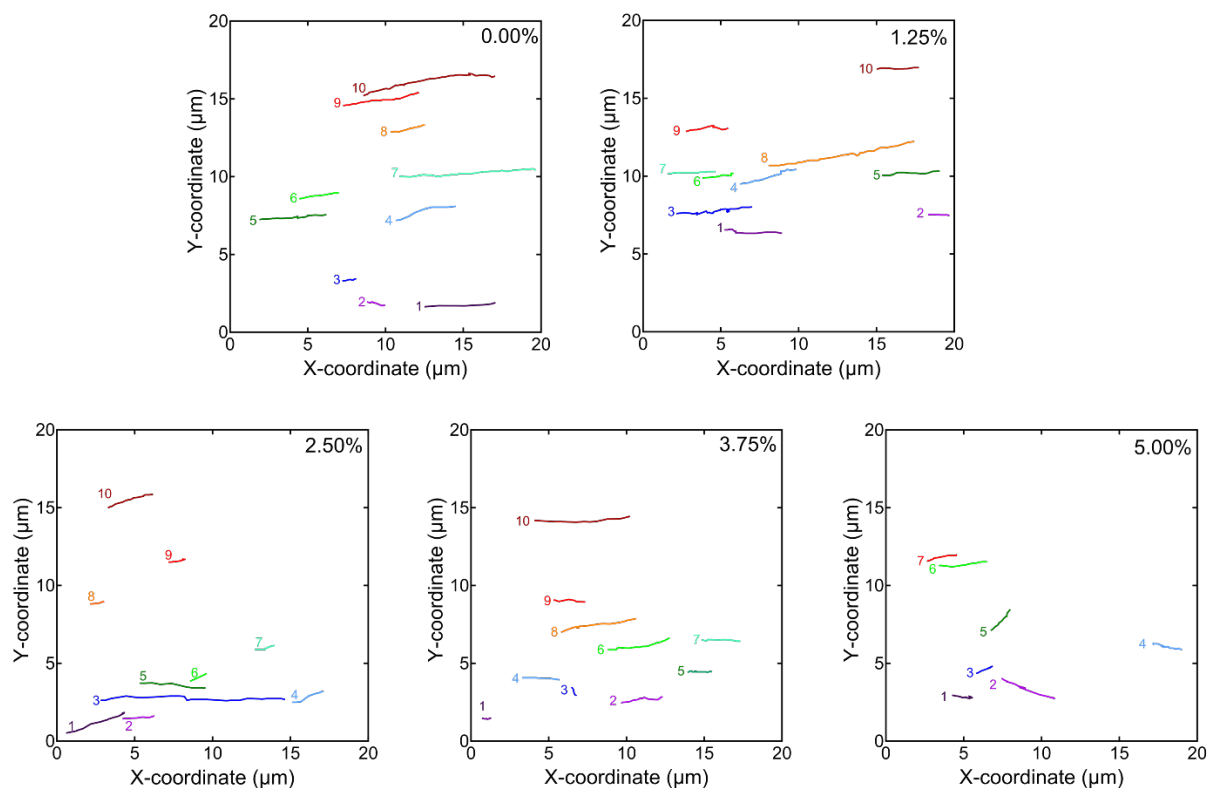
Distributions of the instantaneous gliding velocities of microtubules. The histograms of the instantaneous gliding velocities of microtubules with or without 0.1% glutaraldehyde treatment on kinesin (top panel) and dynein (bottom panel) coated glass substrate are shown. Histograms on the left and right columns are for untreated and glutaraldehyde treated microtubules, respectively. The mean values of the instantaneous gliding velocities with standard deviation, mean $\pm$ SD and the number of microtubules analyzed, n , are given in the legends. Bin size = $0.2 \mu\text{ms}^{-1}$.



Supporting Fig. 4.

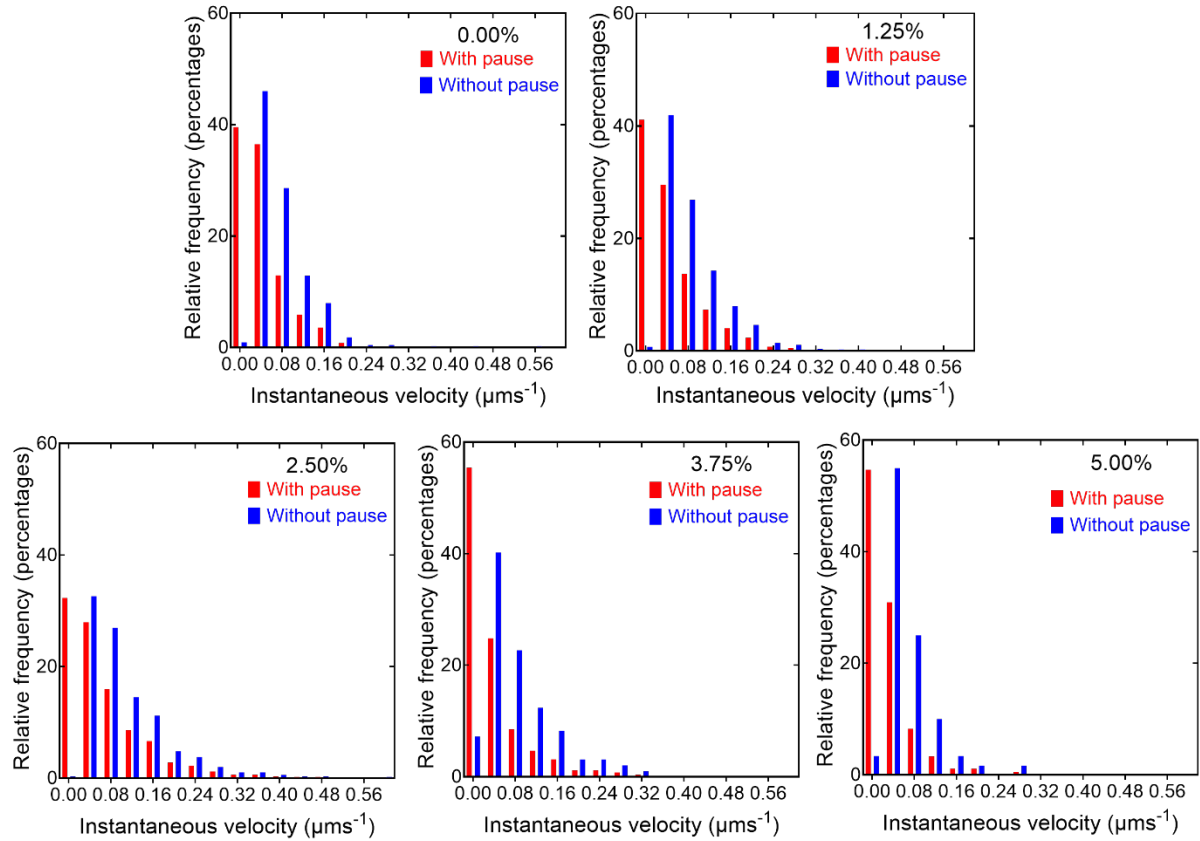
Effect of treatment of microtubules with 0.1% GA on the microtubule-motor protein

interaction. The mean gliding velocities of microtubule with or without 0.1% glutaraldehyde on motor protein coated glass substrate are statistically non-significant as analyzed using Student's t-test. The P-values are 0.3668 and 0.8305, for the case of kinesin and dynein, respectively. ns = non-significant. 20 untreated microtubules and 20 glutaraldehyde-treated microtubules were analyzed for kinesin supported gliding assay and 15 untreated and 16 glutaraldehyde-treated microtubules were analyzed for dynein supported gliding assay. Error bars: standard deviation .



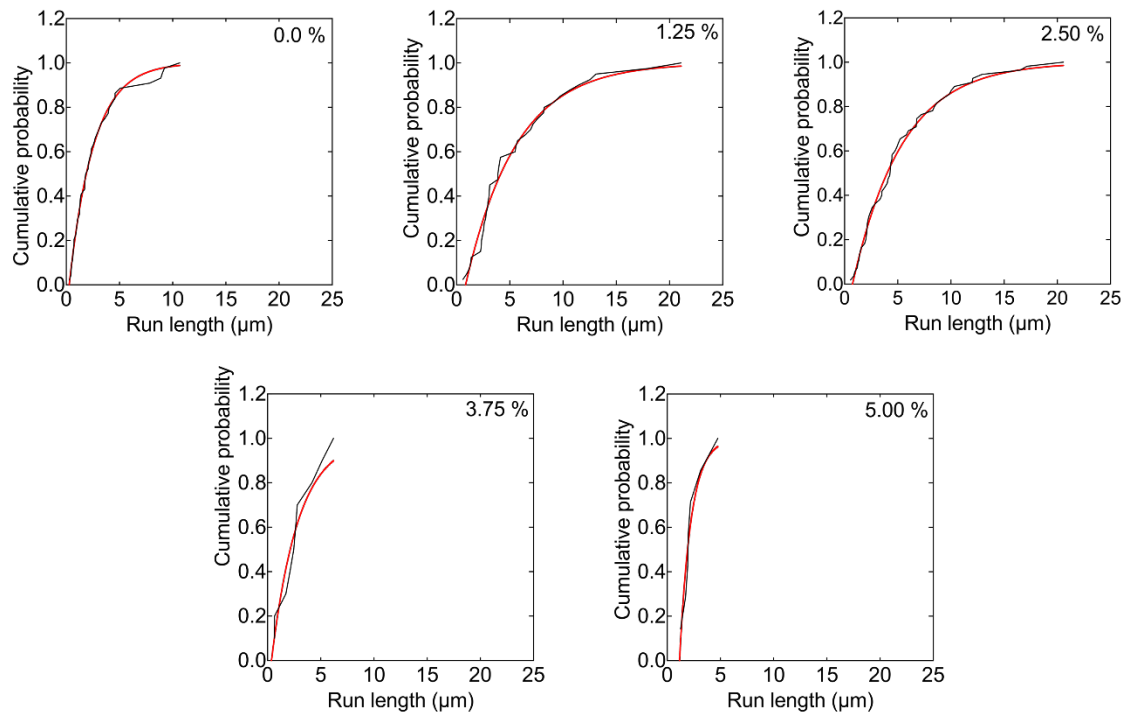
Supporting Fig. 5.

Trajectories of the moving QDs by dynein along microtubules. 10 representative events for each applied tensile strain (7 in the case of 5.00% strain) are shown. The trajectories are numbered according to their coordinates.



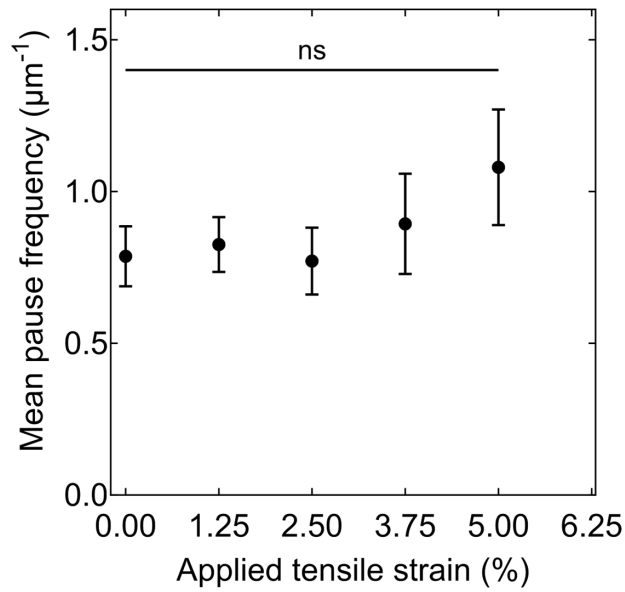
Supporting Fig. 6.

Distributions of the instantaneous velocities of dynein-driven QDs along microtubules stretched under different tensile strains. Distributions of velocities with and without pausing events were plotted. Bin size = $0.04 \mu\text{m s}^{-1}$. The number of QDs considered for this analysis are available in Supporting Table 1.



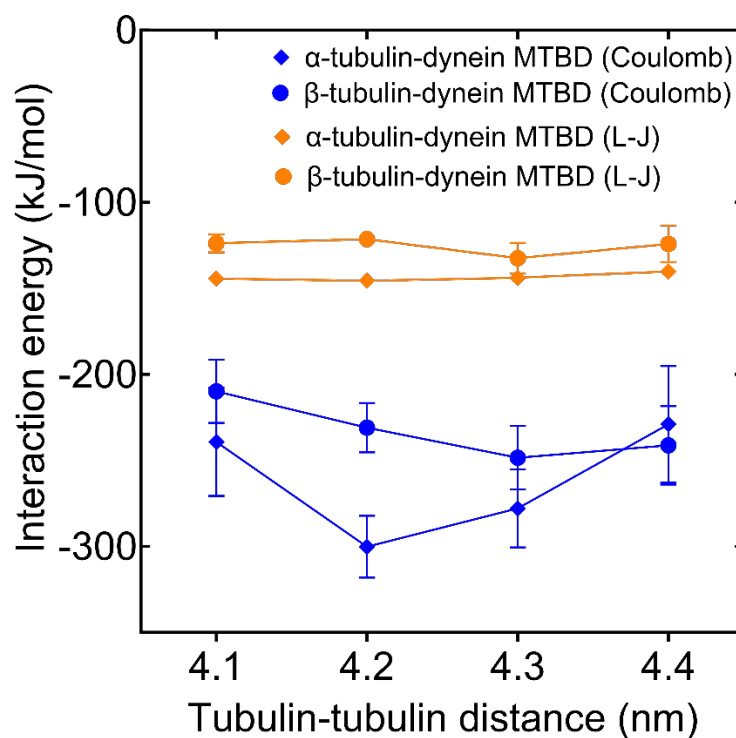
Supporting Fig. 7.

Cumulative frequency distribution of dynein run length at different tensile strains. Mean run length of dynein-driven QD along undeformed and deformed microtubules at different tensile strain was determined using the bin-independent cumulative probability distribution. To determine the run length (x), non-linear least-squares fitting of the cumulative probability distribution to the function: $Y = 1 - \exp\left(\frac{(x_0 - x)}{t}\right)$, was performed. x_0 is the lowest run length measured during analysis. The only fit parameter, the decay constant, t , gave the mean run length in the distribution. Mean values from the fit, the goodness of the fit and the number of QDs analyzed are given in Supporting Table 2.



Supporting Fig. 8.

Effect of applied tensile strain at microtubules on the pause frequency of dynein-driven cargo transport along the microtubules. The difference in the pause frequencies of the QDs transported by multiple dyneins along microtubules under different tensile strains is statistically non-significant when compared with each other using the Kruskal-Wallis test followed by Dunn's test for post hoc comparisons. The number of QDs analyzed for this plot are 44, 40, 55, 10, and 7 at 0.00%, 1.25%, 2.50%, 3.75% and 5.00% tensile strains, respectively. ns = non-significant. Error bar: standard error.



Supporting Fig. 9.

The interaction energies between each of the tubulin monomers and dynein MTBD at different tubulin-tubulin distances. L-J mentioned in the legend stands for Lennard Jones. Five runs were averaged to obtain the mean values. Some error bars cannot be shown as they are shorter than the size of the symbol.

Supporting Table 1**Results of the normality test and the statistical analysis of the velocities of dynein-driven QDs along microtubules at different applied tensile strains.**

The instantaneous velocities were checked for normality using the Kolmogorov-Smirnov normality test. Since all the data were not normally distributed, the Kruskal-Wallis test, followed by Dunn's multiple comparison test was conducted to compare the instantaneous velocities of dynein-driven QDs along microtubules stretched at different tensile strains. Data from Fig. 3b.

Kolmogorov-Smirnov Normality test					
Applied tensile strain (%)	0.00	1.25	2.50	3.75	5.00
KS distance	0.1941	0.2038	0.1952	0.2487	0.2333
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Passed normality test (alpha = 0.001)?	No	No	No	No	No
P value summary	****	****	****	****	****
Number of QDs	44	40	55	10	7
Kruskal-Wallis test					
P value	<0.0001				
Exact or approximate P value?	Approximate				
P value summary	****				
Do the medians vary significantly (P < 0.05)?	Yes				
Number of groups	10				
Kruskal-Wallis statistic	1215				

Dunn's multiple comparisons test				
Applied tensile strain	Mean rank diff.	Significant?	Summary	Adjusted P Value
Comparison among velocities with considering pauses at different tensile strains				
0.00 % vs. 1.25 %	-99.40	No	ns	>0.9999
0.00 % vs. 2.50 %	-621.4	Yes	****	<0.0001
0.00 % vs. 3.75 %	411.5	No	ns	0.1034
0.00 % vs. 5.00 %	541.3	No	*	0.0233
Comparison among velocities without considering pauses at different tensile strains				
0.00 % vs. 1.25 %	-137.5	No	ns	>0.9999
0.00 % vs. 2.50 %	-468.5	No	**	0.0019
0.00 % vs. 3.75 %	125.3	No	ns	>0.9999
0.00 % vs. 5.00 %	345.0	No	ns	>0.9999
Comparison between velocities with and without considering pauses at the same strain				
0.00 % vs. 0.00 %	-1592	Yes	****	<0.0001
1.25 % vs. 1.25 %	-1630	Yes	****	<0.0001
2.50 % vs. 2.50 %	-1439	Yes	****	<0.0001
3.75 % vs. 3.75 %	-1878	Yes	****	<0.0001
5.00 % vs. 5.00 %	-1789	Yes	****	<0.0001

Supporting Table 2

Fitting information of the cumulative probability distribution of run lengths.

Fitting information of the cumulative probability distribution of run lengths of dynein-driven QDs along undeformed and deformed microtubules at different tensile strains (Fig. 3c).

Applied tensile strain (%)	Mean run length (μm)		R^2	Number of QDs
	Mean	Standard Error		
0.00	2.32	0.03	0.9965	44
1.25	4.76	0.16	0.9808	40
2.50	4.69	0.08	0.9934	55
3.75	2.54	0.32	0.9331	10
5.00	1.09	0.19	0.9356	7

Supporting Table 3.**Results of the normality test and the statistical analysis of the run lengths of dynein-driven QDs along microtubules at different applied tensile strains.**

The run lengths were checked for normality using the Kolmogorov-Smirnov normality test. Since all the data were not normally distributed, the Kruskal-Wallis test, followed by Dunn's multiple comparison test was carried out to compare the run lengths of dynein-driven QD transport along microtubules stretched at different tensile strains. Data from Fig. 3d.

Kolmogorov-Smirnov Normality test					
Applied tensile strain (%)	0.00	1.25	2.50	3.75	5.00
KS distance	0.1813	0.2073	0.1739	0.2139	0.2931
P value	0.0009	0.0002	0.0003	>0.1000	0.0699
Passed normality test (alpha = 0.001)?	No	No	No	Yes	Yes
P value summary	***	***	***	ns	ns
Number of values	44	40	55	10	7

Kruskal-Wallis test	
P value	0.0001
Exact or approximate P value?	Approximate
P value summary	***
Do the medians vary significantly ($P < 0.05$)?	Yes
Number of groups	6
Kruskal-Wallis statistic	25.64

Dunn's multiple comparisons test				
Comparison	Mean rank diff.	Significant?	Summary	Adjusted P Value
0.00 vs. 1.25	-38.41	No	**	0.0017
0.00 vs. 2.50	-35.47	No	**	0.0017
0.00 vs. 3.75	-8.532	No	ns	>0.9999
0.00 vs. 5.00	-0.72	No	ns	>0.9999

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